Within-plant variability in blueberry (*Vaccinium corymbosum* L.): maturity at harvest and position within the canopy influence fruit firmness at harvest and postharvest

Gustavo A. Lobos[1*], Claudia Moggia[1,2*], Carolina Bravo[1]; Marcelo Valdés[1], Randolph M. Beaudry[3]; Jordi Graell[2]; Isabel Lara[2]

[1] Plant Breeding and Phenomic Center, Faculty of Agricultural Sciences, Universidad de Talca, Talca, Chile, P.O. Box 747, Talca, Chile.

[2] Unitat de Postcollita-XaRTA, Centre AGROTÈCNIO, Universitat de Lleida, Alcalde Rovira Roure 191, 25198 Lleida, Spain.

[3] Department of Horticulture, Michigan State University, A22 Plant and Soil Sciences Building, Laboratory of Postharvest Physiology, East Lansing, MI 48824, USA.

[*] Corresponding authors at: Universidad de Talca, Facultad de Ciencias Agrarias, 2 Norte 685, Talca, Chile. Tel.: +56 71 2200214; fax: +56 71 2200212; e-mail: globosp@utalca.cl cmoggia@utalca.cl

Abstract

For blueberry, harvest readiness is based on skin color, with fruit being considered ready to pick when the berry skin reaches 100 % blue coverage. The extended bloom period for the blueberry inflorescence and uneven developmental rates, yield 100 % blue fruit that often vary widely in physiological maturity at any given harvest date. The objective of this study was to determine the inherent variability in the firmness of a synchronized cohort of blueberry fruit and the effects of

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harvest delay and position within the canopy on fruit characteristics at harvest and after refrigerated storage. During two seasons, regions of the canopy of ‘Duke’ and ‘Brigitta’ plants were designated as east (E) and west (W) sides. Fruit of a specific developmental stage from each side were either harvested when reaching 100 % blue coverage (ripe fruit: B100) or allowed to stay on the plant for six additional days (over-ripe fruit: B100+6). Despite the narrow period of time elapsed between harvests of ripe and over-ripe fruit, variation in firmness was extensive, with drops up to 24 %, depending on year and cultivar. The six days of additional development were enough to increase the amount of soft and very soft fruit at harvest and after storage, demonstrating the importance of frequent harvests to improve firmness at final destinations. Both the percentage of blue fruit at each harvest date and total fruit produced were higher on the E side of the plant. Year-to-year variation in firmness exceeded that caused by the imposed treatment, which highlights the need to understand the environmental factors contributing to fruit softening. This is the first report on in-plant fruit variability for blueberry and its effect on postharvest performance.

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Keywords: within plant, within canopy, variability, heterogeneity, softening, TSS, TA, ethylene, respiration

1. Introduction

Postharvest performance of fresh blueberries is critical for long-term storage, especially for Chilean fruit, which are exported mainly by boat, typically taking 20 – 50 days to reach final markets and/or consumers (Beaudry et al., 1998; Lobos et al., 2014b; Moggia et al., 2016). Fruit homogeneity is essential to obtain high quality produce, firmness being one of the most critical attributes influencing consumer acceptance (NeSmith et al., 2002). Firmer fruit will better stand harvest and postharvest management (Hanson et al., 1993). Shippers have reported that the rate of non-accepted Chilean fruit at destination has increased during the last decade. Rejections provide evidence of high variability between seasons but also among shipments within a particular season. The root causes of this variability are still uncertain.

The blooming and fruit development periods in northern highbush blueberries usually span 3 – 4 weeks (Retamales and Hancock, 2012) and 42 – 90 days (Darnell, 2006), respectively. Thus fruit will develop under different environmental conditions along the season (Gough, 1994; Lobos et al., 2014a). For instance, first pollinated flowers set fruit that are subjected to lower seasonal temperatures compared to fruit set at the end of the blooming time.

Environmental factors such as temperature and light have important effects on fruit texture (Sams, 1999). Although a cluster fully exposed to sunlight is considered to be growing under enhanced conditions for fruit quality (Smart, 1985), high temperatures could also have negative effects on their metabolism (Bergqvist et al., 2001), indirectly affecting cell structure and other texture-determining components (Vicente et al., 2007). Temperatures higher than 32 ºC during blueberry maturation have been associated with smaller and softer fruit (Mainland, 1989), as well as with reduced anthocyanin production (Prange and DeEll, 1997). Lobos et al. (2013) proved that lower light incidence and temperature contributed to an increase in fruit weight, fruit

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water content, titratable acidity, and firmness, but lead to a decrease in soluble solids content at harvest.

Fruit position within the canopy could represent an additional source of variability. In the main Chilean northern blueberry production area (latitude 35 – 38° S) (Lobos and Hancock, 2015), where orchards are primarily planted in the N-S direction, differences in daily integration of radiation and temperature are expected for leaves and fruit according to their position, either at the east or the west side of the plant. For instance, under high environmental temperatures, the side of the plant that receives lower radiative flux during the afternoon will face a lower gradient in the vapor pressure deficit, favoring stomatal opening and thus having more transpiration than the sunny side of the canopy. The lower radiation, and the lower temperature of the leaf due to transpiration, would reduce losses by photo-oxidation and photo-respiration, respectively, thus increasing the net CO_2 assimilation, and favoring the accumulation of carbohydrates in surrounding fruit (Dale, 1992; Syvertsen et al., 2003).

Accordingly, since most of the physical-chemical fruit characters are influenced by environmental factors (Sams, 1999), it would be expected that species having long blooming period, fast fruit growth and perishable fruits, such as blueberries, will show higher variability in fruit condition at harvest. In addition, harvest index for blueberries is based almost uniquely on skin color, fruit being considered ready to pick when reaching 100 % blue coverage. It is then most likely that within each harvest (typically separated by 6 – 12 d intervals), fruit with similar external appearance, but different physiological maturity, are picked and packed together in the same clamshell.

Concerns regarding over-ripe fruit in packed commercial units started very early in the history of blueberry postharvest research (Bailey, 1947; Woodruff et al., 1960). However, except

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for some initiatives (Vicente et al., 2007; Moggia et al., 2016), this problem has not been fully addressed. Therefore, the objective of this study was to determine the effect of fruit maturity stage at harvest (ripe *vs*. over-ripe) and the possible role of fruit positioning within the canopy (east *vs.* west) on fruit characteristics, especially on firmness, at harvest and after medium and long-term refrigerated storage. Although macro and microclimatic factors were not a part of this study, some basic assessments supporting their importance are included.

2. Material and Methods

2.1.Plant material and trial establishment

Trials were conducted on mature highbush blueberry plants (*Vaccinium corymbosum* L.), cvs. ‘Duke’ and ‘Brigitta’ (9- and 8-year old, respectively), established on a commercial field located in Río Claro, Maule Region - Chile (35° 15’ 33.80’’ S; 71° 14’ 17.70’’ W; 339 m.a.s.l.; N-S orientation: 331.75º), during seasons 2013/14 (Y1) and 2014/15 (Y2). As in most of Chilean commercial orchards, honeybees (*Apis mellifera*) were used as pollinators in a ratio of eight beehives per ha.

At budbreak of Y1, 24 plants of similar characteristics (height, canopy volume, and number and age of canes) were selected for each cultivar, to study the effects of maturity stage and canopy position at the peak of harvest of each commercial picking (~40 – 50 % of annual production) (Supplementary Table 1).

Plants were divided into east (E), top, and west (W) sectors, although fruit were collected only from the E and W sides (Fig. 1). For each canopy side (E and W), fruit were picked at two different maturity stages: (1) ripe fruit: with recent change into 100 % blue coloration (within a maximum of two days) (B100); and (2) over-ripe fruit: berries that, once achieving B100, were left on the plant to ripen for six additional days (B100+6). To identify and isolate fruit of these specific maturities, clusters were initially stripped of all fruit that had greater than 75 % blue coloration. The timing of this step was such that only a small portion (~10 – 15 %) of the total fruit was removed. Fruit color development was monitored until the first fruits in the selected clusters developed 100 % blue coloration. Two days after this point in time, half of 100% blue fruit were harvested (B100) while the other half was picked after another six days on the bush (B100+6). During both seasons, the same elapsed time (6 d) was considered between ripe and over-ripe fruit.

2.2 Fruit evaluations

At each harvest, fruit was characterized in terms of weight (g), firmness (N), total soluble solids (TSS, %), titratable acidity (TA, % citric acid), respiration rate (RR, μg CO_2 kg^{-1} s^{-1}), and ethylene production (EP, ng k^{-1} s^{-1}). After 30 and 45 d of refrigerated storage (0 ºC and 90 – 95 % RH) plus one day at 15 ºC, fruit were analyzed for firmness, TSS, TA and TSS/TA.

Measurements were performed on six replicate samples (four plants ea.) for each treatment combination (position and maturity stage) as follows: (1) fruit weight was measured with an electronic balance (LSV-6200g, Veto y Cía. Ltda., Santiago, Chile) for 25 fruit per replicate; (2) firmness was estimated by a compression device (FirmTech 2, BioWorks Inc., KS, USA), using the maximum slope of the curve as compressive force increased from 15 g to 200 g under a loading rate of 16 mm s^{-1} for 25 fruit per replicate; (3) TSS were assessed with a digital thermo-compensated refractometer (Master–T, Atago, Tokyo, Japan) for five fruit per replicate; (4) TA was determined once per replicate, each one consisting of 10 mL juice diluted (distilled water) to 100 mL and titrated with 0.1 mol L^{-1} NaOH to an end-point pH of 8.2; (5) TSS and TA data were

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used to calculate the TSS/TA ratio; (6) RR was recorded from six replicates of 20 fruit each that were placed in 200 ml sealed glass jars and left in darkness for 2 h at room temperature (18 °C). CO_2 accumulation inside the jars was measured using a gas analyzer (Mocon, Inc., PacCheck 325, Minneapolis, USA). An authenticated standard (2.1 % CO_2 and 2.2 % O_2 in N_2 balance) was used for calibration; and (7) for EP, from the same jars used for RR, a 1 mL gas sample was withdrawn with a syringe from the headspace volume, and injected on a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a 3 mm i.d. column packed with activated alumina, 80/100 mesh. The injector, oven, and detector temperatures were set at 75, 100, and 170 °C, respectively, with helium as the carrier gas (0.67 mL s^{-1}), in the presence of hydrogen and air (0.67 and 6.67 mL s^{-1}, correspondingly). An ethylene standard (1.0 μL L^{-1}) was used for calibration.

2.3 Maturity and productivity on each side of the canopy.

In order to assess the commercial proportion (%) of blue fruit on E and W sides of the canopy during the season, and the total amount of fruit produced (kg per plant) in each case, six additional plants were selected on both cultivars during Y2. Weekly, using the hoop-count technique (Hancock et al., 2000), the percentage of blue fruit by visual assessments was recorded (without distinguishing between B100 and B100+6) and all ripe fruit present on the bush were harvested and weighed.

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2.4 Environmental characteristics.

Using automatic sensors (HOBO S-THB, Onset Computer, Bourne, MA, USA), ambient temperature (ºC) and relative humidity (%) in the field were recorded every 15 minutes, and the information plotted every hour. For each cultivar and season, growing-degree-days (GDD) were calculated by taking the average of daily maximum and minimum temperatures compared to a base temperature of 10 ºC (Jobling and James, 2008). GDD were calculated from date of early green tip to harvest date. Additionally, daily precipitation was recorded using a rain gauge.

2.5 Statistical analyses

For each season (Y1 and Y2) and day of measurement (0, 30 and 45 d of refrigerated storage) data was analyzed using an analysis of variance (ANOVA) for a 2x2 factorial experiment (orientation and maturity stage). When significant differences were found, Tukey's multiple comparison test ($p \leq 0.05$) was applied. Additionally, the Kolmogorov-Smirnov test (D K-S) was used to estimate maximum distances (in absolute values) between cumulative frequency distributions of individual fruit firmness (n = 150); comparisons were performed between orientations (E *vs.* W) for each maturity stage and between maturity stages (B100 *vs.* B100+6) within each orientation. Statistical analyses were carried out using Statgraphics Centurion XVI (v.16.0.09, Statpoint, VA, USA) and R 3.0.0 (R Development Core Team, 2008).

3. Results

3.1 Fruit characteristics at harvest

The firmness frequency distributions of pooled data (maturity stages and orientations) (Fig. 2) indicate that a wide range in firmness existed within each harvest for both cultivars, and also between seasons. For 'Duke', fruit firmness ranged 1.89 – 2.07 N in Y1 and 1.37 – 1.80 N in Y2. 'Brigitta' berries displayed lower firmness values in both seasons ranging 1.56 – 1.89 N in Y1, and 1.32 – 1.55 N in Y2. During Y2, 'Duke' and 'Brigitta' berries had a higher proportion of soft fruit than in Y1 (Fig. 2 and Table 1).

The analysis of variance revealed that for 'Duke' (Table 1), berry orientation on the bush only affected firmness in Y1, firmer fruit coming from the W side of the plant. In contrast maturity stage affected almost all fruit characteristics in both years, with consistent differences as maturity advanced; fruit weight (Y1 and Y2), TSS (Y1), TSS/TA (Y1 and Y2), EP (Y2), and RR (Y2) were higher in over-ripe fruit (B100+6) while firmness (Y1 and Y2), TA (Y1 and Y2), and RR (Y1) were higher on ripe fruit (B100). Two significant interactions (TA and TSS/TA) were found between factors and only in Y1 (Table 1 and Supplementary Table 2); in both cases, only the fruit coming from the E side of the plant experienced significant changes between ripe and over-ripe fruit, decreasing in TA and increasing in TSS/TA.

For 'Brigitta' (Table1), orientation on the plant affected firmness in Y1 (higher at the W side), and EP in Y2 (higher at the E side). Increased fruit maturity (B100+6) reduced fruit weight (Y2), firmness (Y1 and Y2), TA (Y2) and EP (Y1 and Y2), but increased TSS/TA ratios and RR in Y2. Significant interactions occurred only in Y2 for TA and TSS/TA (Table 1 and Supplementary Table 2); although no clear patterns were found through the mean separation (Tukey test), there was a tendency of less differences between B100 and B100+6 on the E side compared to the W side.

3.2 Fruit characteristics after storage

After 30 and 45 d of cold storage, orientation of 'Duke' fruit affected several variables but there was relatively little consistency across years (Table 2). In contrast, the effect of maturity stage was evident, over-ripe fruit (B100+6) being those having lower firmness and TA but higher TSS

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and TSS/TA in almost all the evaluations. Significant interactions were detected only for firmness (Y1, 30 d) (Table 2 and Supplementary Table 2); but no clear patterns were found when mean separation was determined by Tukey test.

Similar to 'Duke', and with the exception of firmness (Y1, 45 d; higher on E side), analysis of variance for fruit orientation on 'Brigitta' bushes had little impact on characteristics of stored fruit (Table 2). Again, the effect of maturity stage at harvest was more pronounced, with less mature berries (B100) being higher in firmness and TA, but lower in TSS and TSS/TA. No interactions were detected in this cultivar (Table 2).

3.3 Firmness cumulative frequency distributions at harvest and after storage

The D K-S analysis of cumulative frequencies curves for fruit firmness (Figs. 3 and 4, and Table 3) allowed a more comprehensive characterization of the differences originating from fruit orientation and maturity stage at harvest. Under this approach, it is possible to compare treatments according to different firmness classes for a given cumulative frequency (e.g., 50 % represented by a horizontal dashed line on Figs. 3 and 4) or to look at the proportion of fruit being equal to or lower than a given firmness threshold (e.g., 2 N represented by a vertical dashed line on Figs. 3 and 4). As previously described, Y2 fruit were softer regardless of cultivar, orientation or maturity stage, since these samples displayed in all cases larger percentages of fruit with firmness values < 2 N in comparison with Y1 produce (Figs. 3 and 4). At harvest very soft fruit (< 1.4 N) varied between 0 – 31 % for Y1 and 11 – 67 % depending on cultivar, maturity stage and orientation.

When 'Duke' fruit were compared under the same maturity stage (B100 or B100+6), the effect of orientation (E *vs*. W) was significant only during Y1 at 0 d, B100 and B100+6 having

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lower firmness on the E side (Fig. 3A and Table 3) and at 30 d, B100+6 fruit being softer on the E side (Fig. 3B and Table 3). When comparisons were performed under the same orientation (E or W), the differences between maturity stages (B100 *vs*. B100+6) were evident (Fig. 3), with a greater proportion of firmer fruit at the B100 stage; significant distances (Table 3) between maturity stages were detected at: Y1: 0 d (E side; Fig. 3A), 30 d (E side; Fig. 3B), and 45 d (E and W sides; Fig. 3C); and Y2: 0, 30 and 45 d for fruit from both E and W sides of the bush (Figs. 3D – F).

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The frequency curve differences observed for 'Brigitta' were less marked than those for 'Duke' (Fig. 4 and Table 3). Under the same maturity stage (B100 or B100+6), the effect of orientation (E *vs*. W) was significant only for Y1 (0 and 45 d); E side produced softer B100 fruit at day 0 (Fig. 4A and Table 3), while W side generated softer B100+6 fruit at 45 d (Fig. 4C and Table 3). When comparisons were performed for a given orientation (E or W), the differences between maturity stages (B100 *vs*. B100+6) varied according to the year, although more mature fruit (B100+6) were typically softer than ripe fruit (B100). Significant distances (Table 3) between maturity stages were detected at 0 d (E and W sides; Fig. 4A and Table 3) and after 45 d during Y1 (E side; Fig. 4C and Table 3), and at 0, 30 and 45 d for fruit from both E and W sides of the plant during Y2 (Figs. 4D – F).

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3.4 Maturity and productivity on each side of the canopy.

For each cultivar and evaluation date, more blue fruit were produced from the E than from the W sides of the canopy (Fig. 5A). 'Duke' and 'Brigitta' plants displayed similar percentages of 100 % blue fruit, both at the beginning (~80% E and ~20% W) and at the end of the season (~50% on each side). Productivity followed the same trend, the E side producing more kilograms of fruit per plant than the W side (Fig. 5B). Additionally, ‘Duke’ plants produced more fruit per plant than ‘Brigitta’ (4.2 and 3.1 kg *vs*. 3.6 and 2.2 for the E and the W sides of the canopy, respectively).

3.5 Environmental characteristics.

The Y1 season registered higher temperatures (Fig. 6 A) and lower relative humidity (Fig. 6 B) along the whole period of fruit development from early green tip up to harvest. The Y2 season had higher daytime temperatures and lower relative humidities later in the day, especially for dates close to harvest. Total rainfall was also higher for Y2 than for Y1, with significant rain events (>5 mm) after early bloom and before the harvest of ‘Brigitta’ berries (Fig. 6 C). The GDD accumulation from early green tip to harvest (B100) was 338 and 315 for ‘Duke’ (Y1 and Y2, respectively), whereas ‘Brigitta’ on Y1 and Y2 achieved 582 and 542 GDD, correspondingly (Supplementary Table 1). Increase in GDD between B100 and B100+6 was of 31 and 32 for ‘Duke’ (Y1 and Y2, respectively), and of 42 and 36 for ‘Brigitta’ on the consequent years (Supplementary Table 1).

4. Discussion

Several studies, primarily on apples, have reported that multiple sources of variation, both between-plants and especially within-plant can be found, resulting in heterogeneous quality of harvested fruit (Heinicke, 1966; Jackson, 1967; Robinson et al., 1983; Perring, 1989; Broom et al., 1998; De Silva et al., 2000). Despite the relevance of fruit quality consistency for consumer acceptance, no formal reports have been published for blueberries.

The high variability in firmness of harvested blueberries having similar external characteristics in the current study (6-d interval between B100 and B100+6 fruit), regardless of cultivar or season (Y), is troublesome. The broad range in blueberry fruit firmness is consistent with the report by Moggia et al. (2017), who found that percentages of very soft fruit (firmness < 1.4 N) varied between 25 and 42 % for 'Duke', and between 5 and 10 % for 'Brigitta' in two consecutive years. Blueberry firmness variability is higher than for other fruit species such as apple, where very soft fruit (58 – 62 N) represent less than 0.5 – 0.8% of the total batch harvested (Herregods and Goffings, 1993; De Silva et al., 2000).

When Moggia et al. (2017) segregated fruit by firmness at harvest, it was suggested that fruit within the soft category (<1.60 N) corresponded to those staying longer in the plant after turning completely blue. In the present study, the comparison of fruit quality traits between B100 and B100+6 at harvest, confirms that fruit having lower firmness and TA, along with higher TSS and TSS/TA were primarily associated to the over-ripe harvesting (B100+6). This highlights the importance of maturity stage at harvest, especially for blueberries subjected to long distance shipments. Additionally, the differences on fruit firmness found between Y1 and Y2 also emphasize the importance of climatic conditions during fruit growth and maturation. This reinforces the idea that elapsed time between harvests should be based on physiological changes and environmental conditions rather than a fixed interval. Unfortunately, it is a common practice for growers to wait for blue fruit to accumulate in the bush in order to optimize harvesting labor management and associated costs; in general, the 6 d interval used in this study could be considered as the lowest commercial gap between harvests.

According to GDD, during Y2 both cultivars were harvested having lower unit grades, and, consequently, firmer fruit would have been expected compared to Y1. Nevertheless, data

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from ANOVA and accumulated frequencies proved that fruit was softer during Y2. Since GDD is only a heat index closely related to fruit maturation, this finding highlights the relevance of other environmental factors (e.g., extreme high temperatures, rain close to harvest) influencing fruit conditions at harvest and during cold storage, as discussed later.

The lack of interactions between maturity and fruit position within the canopy would suggest that fruit ripen evenly on both E and W sides of the bush. For perspective, however, it is worth noting that in experiments with a factorial design, the average of each level within one factor is calculated considering the combination of the levels of the other factor (Lawal, 2014). Therefore, the much bigger effects due to maturity stage (B100 *vs*. B100+6) as compared to those arising from orientation (E *vs*. W) could be masking the possible canopy side effect. These results, which are probably also influenced by the high variability found within the samples, suggest that ANOVA procedures, which are based on mean values, might be not necessarily the best approach for finding differences in fruit firmness associated to a particular orientation. Hence, the analysis of the cumulative frequency distribution of all data was used as an alternative approximation. This approach not only allowed the comparison between treatments, but also the visualization of detailed information, such as different firmness categories and the percentage in which each was present. Despite the advantages and disadvantages of both methodologies, results illustrate that for blueberries, a wide range of firmness can be found at a particular harvest, with the consequent risks associated to the presence of over-ripe fruit within commercial units (Bailey, 1947; Woodruff et al., 1960; Moggia et al., 2016 and 2017). This study suggests that the proportion of over-ripe fruit, influenced by the time elapsed between pickings along with particular environmental conditions, that need to be defined in future studies, act in a complementary way to determine the postharvest life of fruit within a particular shipment. Based

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on this, it can be expected that picking frequency would influence the proportion of ripe and over-ripe fruit within the clamshell, whereas environmental conditions would determine postharvest potential of each maturity class.

Although variation in firmness associated to the position of the fruit within the canopy at a given maturity stage (B100 or B100+6) was less marked, still some differences were found between E and W sides. Due to the relatively inefficient water-conducting system of blueberry plants (Gough, 1994), when transpirational demand exceeds capacity, blueberry fruit would be under stress during part of the day (Chen et al., 2012; Estrada et al., 2015). Thus, these orientation-related differences could be explained, in part, by the daily macro- (e.g., ambient temperature, relative humidity, and water availability) and micro-climatic (within each canopy side) fluctuations, which might be also affecting fruit condition at harvest and during postharvest during the whole season. Even though the influence of preharvest environmental conditions on postharvest behavior of fruit is still not well understood for this species, it is known that optimal temperatures for gas exchange are around 20 – 25 ºC (Davis and Flore, 1986); leaf temperatures would be associated to the canopy cooling capacity during the afternoon and to the generation of carbohydrates for fruit growth and development.

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Since both cultivar plots were N-S oriented, fruit from the W side of the bush would be subjected to direct radiative flux during the afternoon (higher ambient temperatures and lower relative humidity; Fig. 6A and B), raising leaf and fruit temperature on that side along the season. Because of this, there is not a clear explanation for the E side of the canopy having softer fruit in some of the measurements. Other morphological and anatomical changes during fruit development may be responsible for firmness and shelf life behavior; a progressive increase in thickness of the epicuticular wax layer and cuticle as well as in the cell walls of the epidermis and hypodermis has been reported in blueberries (Konarska, 2015). While many of these qualitative traits have a genetic background, they would also depend, to a great extent, on environmental conditions and maturity stage at harvest (Connor et al., 2002).

Interestingly, significant differences in firmness between plant sides were only detected for Y1, when values for B100 and B100+6 fruit at harvest were higher than those observed in Y2. Orientation-related differences in fruit firmness remained during postharvest evaluations of B100+6 fruit, both after 30 and 45 d of storage, suggesting that depending on firmness distribution at harvest and environmental conditions, fruit position might be a source of variability. Therefore, in order to integrate harvest by orientation as a useful tool for the grower, the firmness threshold at which the side of the plant may become a relevant postharvest factor must be studied in greater depth.

Variation in fruit firmness between seasons has been previously reported (Ehlenfeldt and Martin, 2002), and it was suggested that softer fruit might be linked to average to above-average rainfall patterns. Pritts and Hancock (1992) stated that rain during harvest can adversely affect fruit quality of highbush blueberries by delaying harvest, washing off fungicides, softening berries, moistening stem scars, and splitting berries. High temperature combined with rain exacerbates these problems. The second season considered in this study (Y2) had more rain, not only at the beginning of the season (Aug. – Sep.) but also during the maturation and harvest periods. Environmental information for both seasons suggests that even the length of each raining event would influence berry softening. In comparison to Y1, Y2 also displayed higher temperatures and lower relative humidity later in the day at near-harvest dates, which would represent another possible source of variability between seasons.

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Environmental conditions could also be responsible for the difference in fruit productivity found between the E and W sides of the canopy. Although productivity was measured only in one year, results are consistent for both cultivars. No measurements were taken at the beginning of the season in order to determine whether the E side started with more or with better quality (higher number of flowers per cluster) flower buds, but pollination dynamics could be a possible explanation for these canopy asymmetry. Honeybees modify their foraging visits according to the nectar production rhythms (Moore et al., 1989), and may use volatile floral emissions (attractant and repellent) as information to regulate their activity by assessing the quality of flowers prior to the contact (Dobson, 2006; Raguso, 2008). According to Rodríguez-Saona (2011), after bees visit and pollinate highbush blueberry flowers, the production of nectar is reduced, concomitantly with relatively predictable changes in the emission of particular volatiles. The same authors also found that the amount of volatiles released were two-fold greater between 09:00 and 12:00 h than during earlier or later periods of the day. Additionally, nectar production is also higher between 09:00 and 11:00 h. Honeybee foraging has a lower ambient temperature threshold of 12 to 14 ºC (Winston, 1987). In the morning, the E side of the plant is more fully illuminated and so it is warmer, and hence volatiles and nectar rewards are at their maximum. This suggests that within orchards oriented in the N-S direction, conditions may favor pollination and fruit set on the E side of the plant.

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5. Conclusions

Frequency analysis of firmness within a well-defined maturity stage (B100 or B100+6) demonstrates that a high degree of variability exists within populations of blueberry fruit related to cultivar, season and orientation on the bush. In addition to these factors, fruit maturity stage

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contributes meaningfully to the overall variation expected in typical commercial harvests. Although the reasons for the high degree of firmness variation within each picking date are not well understood, harvest index based almost uniquely on skin color and the fast evolution of fruit maturation (physiological age) between harvests would be responsible for the wide rage of firmness on each picking. This study shows that even the short time elapsed between B100 and B100+6 stages (6 d) was enough to increase the amount of soft (1.4 – 1.6 N) and very soft fruit (< 1.4 N), and makes clear the importance of more frequent harvests to improve firmness at final destinations, especially when preharvest environmental conditions could accelerate fruit softening as they did in Y2 of this study.

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Even though the effect of fruit position in the canopy was less consistent than that of maturity stage, cumulative frequency distributions suggest that the E side of the plant would produce softer fruit. Data also support the idea that, within the same picking date, export-destined clamshells would contain a greater proportion of fruit coming from the E side of the canopy. Since differences between canopy sides were only detected in Y1, results also suggest that there is a firmness threshold above which differences among orientations can be found.

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Increasing temperatures and weather variability expected with climate change indicate that blueberry growers will face an increase in the already high variability in fruit firmness. It will be therefore critical to establish the main environmental factors contributing and predisposing fruit to softening.

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Author Contributions

Gustavo A. Lobos, Claudia Moggia, Randy Beaudry, Isabel Lara and Jordi Graell contributed to the conception and design of the work. Carolina Bravo, Marcelo Valdes, Claudia Moggia, Randy Beaudry and Gustavo A. Lobos performed acquisition, analysis, and interpretation of data for the work. Gustavo A. Lobos, Claudia Moggia, Carolina Bravo, Randy Beaudry, Isabel Lara and Jordi Graell collaborated to generate and validate the version to be published.

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Tables

Table 1. Analysis of variance of fruit quality traits[z] for ‘Duke’ and ‘Brigitta’ berries coming from different orientations (east and west) and picked at different maturity stages (B100 and B100+6). Fruit were harvested and assessed at the peak of harvest of seasons 2013/14 (Y1) and 2014/15 (Y2).

		DUKE							BRIGITTA						
		W (g)	F (N)	TSS (%)	TA (%)	TSS/TA	EP (ng kg^{-1} s^{-1})	RR (µg CO_2 kg^{-1} s^{-1})	W (g)	F (N)	TSS (%)	TA (%)	TSS/TA	EP (ng kg^{-1} s^{-1})	RR (µg CO_2 kg^{-1} s^{-1})
Y1	**Orientation (O)**														
	East	1.81	1.92 b	16.8	0.79	24.6	0.49	33.58	1.73	1.67 b	16.2	0.84	20.5	0.05	20.58
	West	1.79	2.10 a	16.5	0.79	21.9	0.46	32.48	1.72	1.78 a	16.2	0.79	21.1	0.05	19.22
	Significance (p)	*0.3539*	***0.0000***	*0.7119*	*0.9704*	*0.3291*	*0.5772*	*0.3156*	*0.6294*	***0.0000***	*0.9633*	*0.5054*	*0.7231*	*0.8914*	*0.4197*
	Maturity (M)														
	B100	1.69 b	2.07 a	15.6 b	0.96 a	16.5 b	0.48	37.53 a	1.70	1.89 a	16.2	0.82	20.9	0.07 a	20.67
	B100+6	1.90 a	1.95 b	17.6 a	0.62 b	30.1 a	0.48	28.52 b	1.74	1.56 b	16.1	0.81	20.7	0.03 b	19.13
	Significance (p)	***0.0000***	***0.0012***	***0.0002***	***0.0004***	***0.0001***	*0.9558*	***0.0000***	*0.2510*	***0.0000***	*0.8996*	*0.8298*	*0.9243*	***0.0129***	*0.1283*
	O x M														
	Significance (p)	*0.9355*	*0.8124*	*0.2212*	***0.0322***	***0.0213***	*0.3117*	*0.2821*	*0.8390*	*0.0669*	*0.4215*	*0.7913*	*0.8294*	*0.2242*	*0.7730*
Y2	**Orientation (O)**														
	East	1.60	1.57	15.0	0.66	23.8	0.26	14.28	1.77	1.44	15.5	0.65	26.5	0.07 a	7.28
	West	1.61	1.59	14.8	0.66	23.6	0.23	14.33	1.79	1.43	14.9	0.59	30.4	0.06 b	7.26
	Significance (p)	*0.8089*	*0.5568*	*0.7387*	*0.9483*	*0.9572*	*0.8318*	*0.9227*	*0.7548*	*0.6608*	*0.2583*	*0.7519*	*0.9587*	***0.0091***	*0.6502*
	Maturity (M)														
	B100	1.52 b	1.80 a	14.8	0.76 a	19.7 b	0.12 b	12.43 b	1.92 a	1.55 a	14.9	0.82 a	18.5 b	0.08 a	5.20 b
	B100+6	1.70 a	1.37 b	15.1	0.56 b	27.7 a	0.38 a	16.18 a	1.64 b	1.32 b	15.5	0.43 b	38.4 a	0.06 b	9.35 a
	Significance (p)	***0.0000***	***0.0000***	*0.5237*	***0.0040***	***0.0080***	***0.0000***	***0.0016***	***0.0000***	***0.0000***	*0.2097*	***0.0000***	***0.0000***	***0.0280***	***0.0001***
	O x M														
	Significance (p)	*0.9891*	*0.2368*	*0.0598*	*0.9344*	*0.4540*	*0.1957*	*0.1627*	*0.3514*	*0.3169*	*0.4470*	***0.0018***	***0.0024***	*0.8397*	*0.2933*

[z] Traits: fruit weight (W), firmness (F), TSS (total soluble solids), TA (titratable acidity), ethylene production (EP), and respiration rate (RR). Mean separation by Tukey test ($p \leq 0.05$).

Table 2. Analysis of variance of fruit quality traits[z] for 'Duke' and 'Brigitta' berries coming from different orientations (east and west) and picked at different maturity stages (B100 and B100+6). Fruit were harvested at the peak of the seasons 2013/14 (Y1) and 2014/15 (Y2), and assessed after 30 and 45 days under cold storage.

		F (N)				TSS (%)				TA (%)				TSS/TA			
		DUKE		BRIGITTA		DUKE		BRIGITTA		DUKE		BRIGITTA		DUKE		BRIGITTA	
		30	45	30	45	30	45	30	45	30	45	30	45	30	45	30	45
Y1	**Orientation (O)**																
	East	2.07 b	1.87	1.52	1.58 a	17.6	15.4	15.2	14.7	0.69	0.69 b	0.69	0.71	26.3	25.5 a	23.7	21.7
	West	2.23 a	1.87	1.61	1.44 b	17.4	15.0	15.3	14.9	0.73	0.76 a	0.71	0.67	24.7	21.2 b	21.4	24.4
	Significance (p)	***0.0039***	*0.9139*	*0.0691*	***0.0088***	*0.6632*	*0.1703*	*0.8656*	*0.3100*	*0.5544*	***0.0447***	*0.2072*	*0.1608*	*0.5273*	***0.0039***	*0.5000*	*0.4593*
	Maturity (M)																
	B100	2.18	2.18 a	1.53	1.53	16.1 b	14.6 b	15.3	14.4 b	0.78 a	0.92 a	0.72	0.76	21.1 b	16.0 b	21.2	20.0 b
	B100+6	2.12	1.56 b	1.60	1.49	18.9 a	15.7 a	15.2	15.2 a	0.65 b	0.52 b	0.67	0.62	29.8 a	30.7 a	23.9	26.1 a
	Significance (p)	*0.1010*	***0.0000***	*0.0941*	*0.9203*	***0.0000***	***0.0006***	*0.8232*	***0.0020***	***0.0494***	***0.0010***	*0.5178*	*0.0620*	***0.0023***	***0.0002***	*0.4226*	***0.0254***
	O x M																
	Significance (p)	***0.0333***	*0.4834*	*0.1843*	*0.4293*	*0.1337*	*0.2604*	*0.5038*	*0.4388*	*0.9445*	*0.4391*	*0.6609*	*0.4576*	*0.6292*	*0.0587*	*0.6667*	*0.7028*
Y2	**Orientation (O)**																
	East	1.64	1.86	1.72	1.62	13.9	15.1 b	15.9	14.7	0.66 a	0.60	0.49	0.55	21.9 b	26.1	33.6	28.2
	West	1.62	1.94	1.68	1.66	14.1	16.0 a	16.2	14.7	0.50 b	0.64	0.51	0.56	32.6 a	26.1	32.7	28.2
	Significance (p)	*0.4026*	*0.0876*	*0.2393*	*0.3101*	*0.5925*	***0.0091***	*0.4535*	*0.8836*	***0.0214***	*0.7101*	*0.7212*	*0.8290*	***0.0013***	*0.9973*	*0.8286*	*0.9784*
	Maturity (M)																
	B100	1.72 a	2.00 a	1.87 a	1.79 a	12.3 b	15.1 b	16.3	14.9	0.66 a	0.63	0.57 a	0.68 a	18.7 b	24.5	29.7 b	22.8 b
	B100+6	1.54 b	1.79 b	1.53 b	1.49 b	15.8 a	16.0 a	15.8	14.6	0.49 b	0.60	0.43 b	0.44 b	35.8 a	27.8	36.5 a	33.6 a
	Significance (p)	***0.0000***	***0.0003***	***0.0000***	***0.0000***	***0.0000***	***0.0489***	*0.2147*	*0.3493*	***0.0105***	*0.5627*	***0.0247***	***0.0006***	***0.0013***	*0.1628*	***0.0493***	***0.0029***
	O x M																
	Significance (p)	*0.2957*	*0.2352*	*0.6103*	*0.4328*	*0.3378*	*0.6187*	*0.0586*	*0.5213*	*0.1647*	*0.1034*	*0.6803*	*0.9635*	*0.0844*	*0.0970*	*0.5382*	*0.7436*

[z] Traits: firmness (F), TSS (total soluble solids), and TA (titratable acidity). Mean separation by Tukey test ($p \leq 0.05$).

Table 3. Firmness cumulative frequency distance (absolute values) and significance of Kolmogorov-Smirnov analysis for ‘Duke’ and ‘Brigitta’ berries coming from different orientations (east and west) and maturity stages (B100 and B100+6). Fruit were harvested at the peak of the seasons 2013/14 (Y1) and 2014/15 (Y2), and assessed at harvest (0 d), and after 30 (30 d) and 45 (45 d) days under cold storage.

Cultivar	**Factor**	**Level**	**Comparison**	**Y1**			**Y2**		
				0 d	30 d	45 d	0 d	30 d	45 d
DUKE	Maturity	B100	East *vs*. West	0.33 ***[z]	0.15 n.s.	0.09 n.s.	0.14 n.s.	0.17 n.s.	0.13 n.s.
		B100+6	East *vs*. West	0.35 ***	0.38 ***	0.11 n.s.	0.16 n.s.	0.09 n.s.	0.16 n.s.
	Orientation	East	B100 *vs*. B 100+6	0.24 **	0.22 **	0.56 ***	0.44 ***	0.40 ***	0.34 ***
		West	B100 *vs*. B 100+6	0.20 n.s.	0.16 n.s.	0.45 ***	0.63 ***	0.26 **	0.29 ***
BRIGITTA	Maturity	B100	East *vs*. West	0.40 ***	0.22 n.s.	0.23 n.s.	0.12 n.s.	0.08 n.s.	0.06 n.s.
		B100+6	East *vs*. West	0.16 n.s.	0.11 n.s.	0.19 *	0.06 n.s.	0.09 n.s.	0.07 n.s.
	Orientation	East	B100 *vs*. B 100+6	0.27 ***	0.13 n.s.	0.23 **	0.48 ***	0.44 ***	0.37 ***
		West	B100 *vs*. B 100+6	0.49 ***	0.08 n.s.	0.15 n.s.	0.42 ***	0.45 ***	0.31 ***

[z] (n.s.: non-significant; *: $p < 0.05$; **; $p < 0.01$; and ***: $p < 0.001$).

Figures

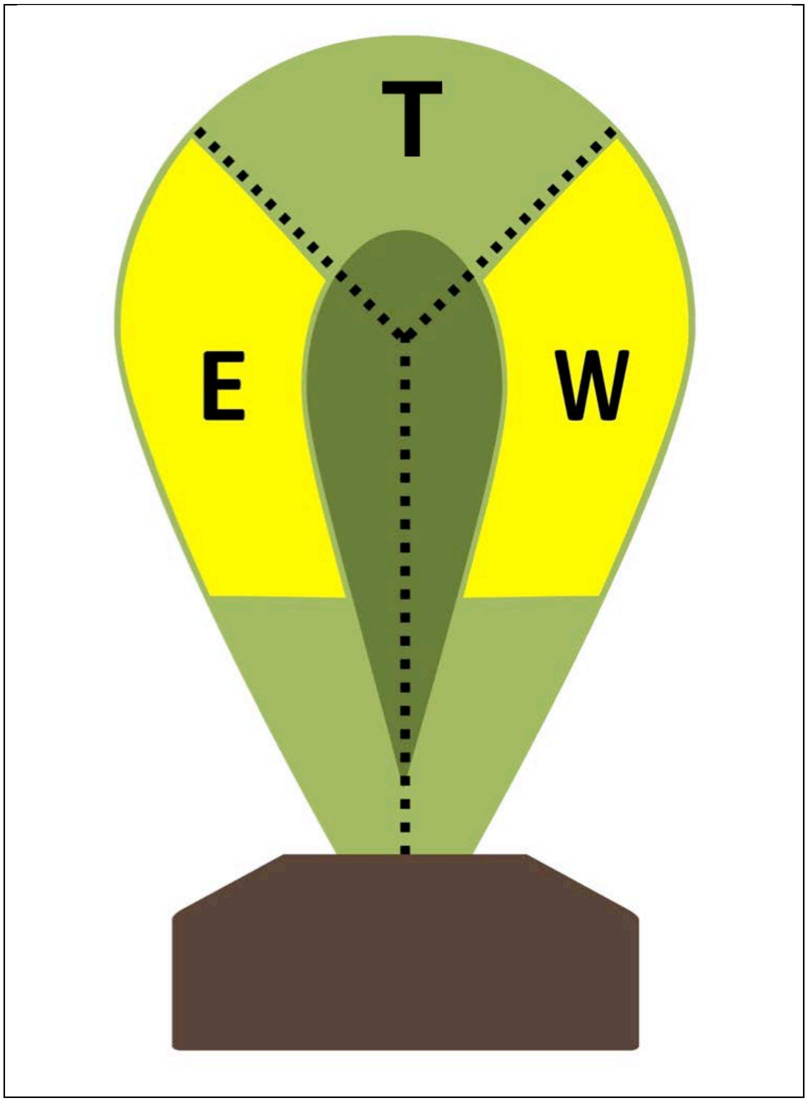

Figure 1. Scheme of bush segmentation into east (E), top (T) and west (W) orientation. On E and W side, delimited by dashed lines, fruit was harvested from the yellow area.

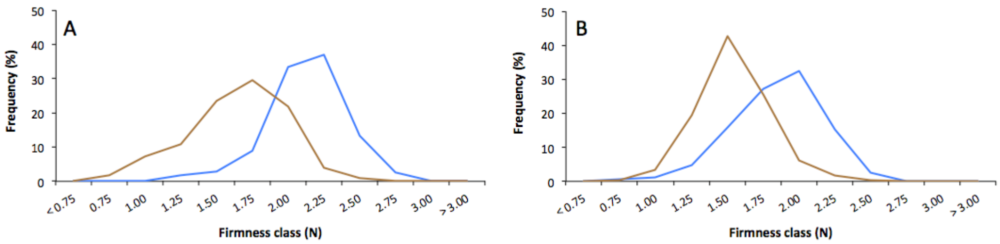

Figure 2. Frequency distribution of firmness classes of ‘Duke’ (A) and ‘Brigitta’ (B) fruit picked at the peak of harvest of seasons 2013/14 (Y1; blue lines) and 2014/15 (Y2; brown lines). Each line represents the combination of both fruit position (east and west) and maturity stage (ripe and over-ripe).

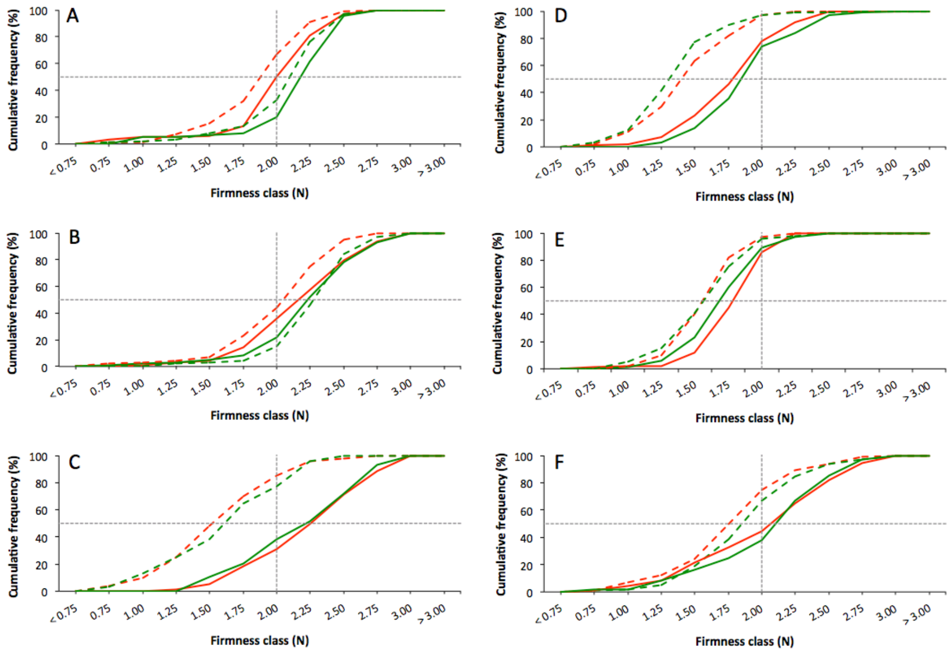

Figure 3. Firmness (N) cumulative frequency distribution of 'Duke' harvested at the peak of seasons 2013/14 (A, B and C) and 2014/15 (D, E and F). Measurements taken at harvest (A and D), and after 30 (B and E) and 45 (C and F) days of cold storage. Berries were picked from East (red lines) and West (green lines) side of the plant, when fruit reached 100% blue color within a maximum of 2 days (B100: solid lines) or left remaining on the plant for additional 6 days (B100+6: dashed lines). Each graph includes a horizontal (50% of the cumulative frequency) and vertical (firmness class at 2 N) dashed lines, only for reference purposes. n = 150 fruit.

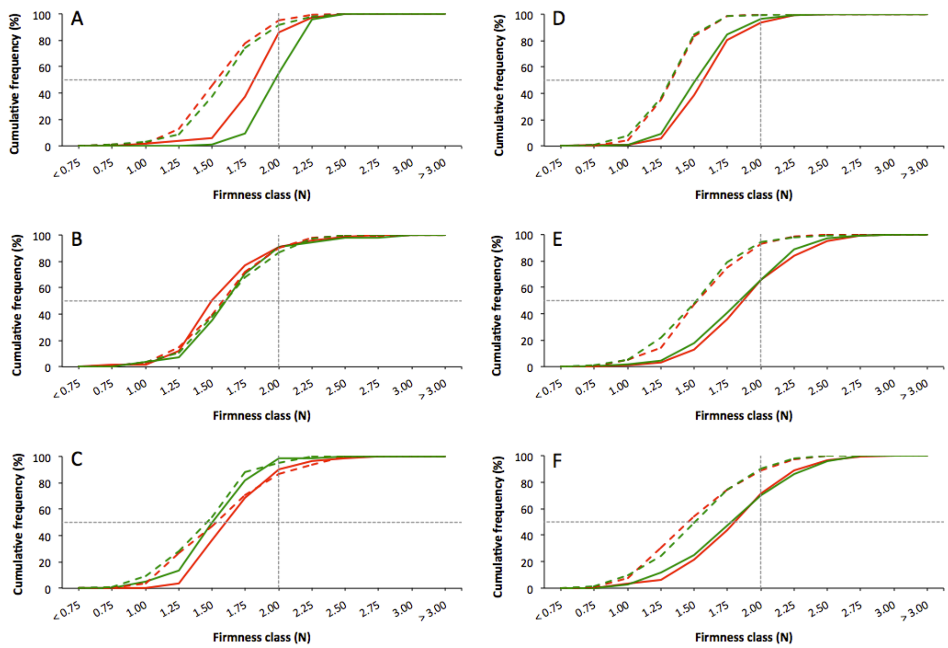

Figure 4. Firmness (N) cumulative frequency distribution of 'Brigitta' harvested at the peak of seasons 2013/14 (A, B and C) and 2014/15 (D, E and F). Measurements taken at harvest (A and D), and after 30 (B and E) and 45 (C and F) days of cold storage. Berries were picked from East (red lines) and West (green lines) side of the plant, when fruit reached 100% blue color within a maximum of 2 days (B100: solid lines) or left remaining on the plant for additional 6 days (B100+6: dashed lines). Each graph includes a horizontal (50% of the cumulative frequency) and vertical (firmness class at 2 N) dashed lines, only for reference purposes. n = 150 fruit.

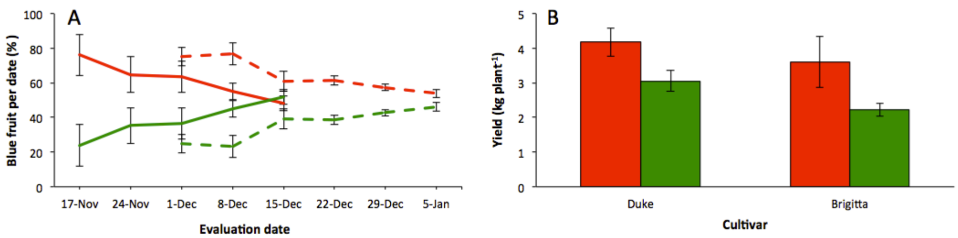

Figure 5. Weekly proportion of 100% blue fruit (A; ‘Duke’ with solid lines and ‘Brigitta’ with dashed lines) and total amount of fruit (B) produced by the East (red lines and bars, respectively) and West (green lines and bars, respectively) sides of the canopy during season 2014/15. Vertical black bars indicate standard error.

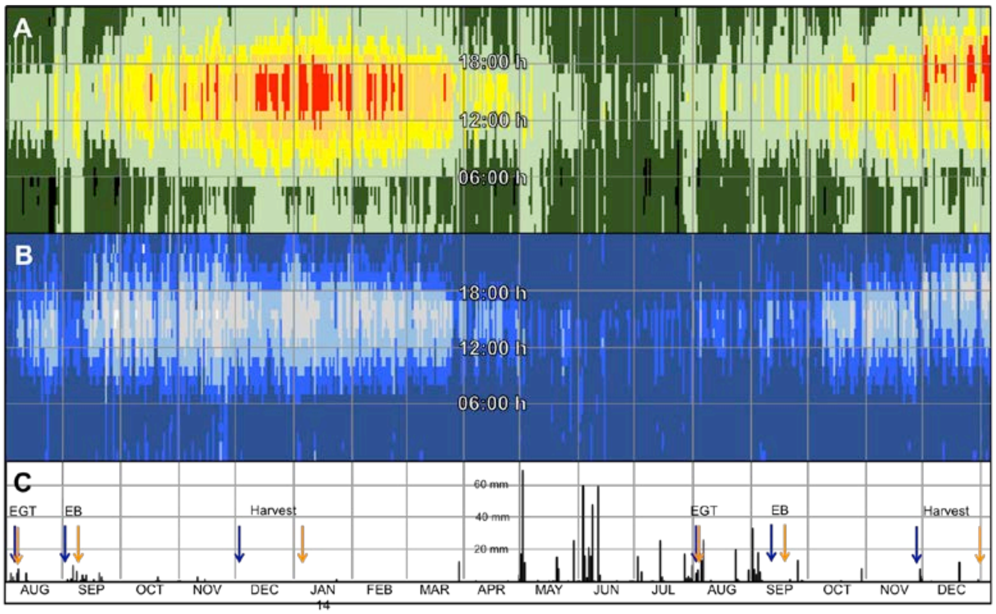

Figure 6. Hourly ambient temperature (A) and relative humidity (B) bands, and daily precipitation (C), from August 01, 2013 until January 05, 2015. Data is color coded by temperature range (ºC) (black: < 0; dark green: 0–10; light green: 10–18; yellow: 18–24; orange: 24–29; and red: 29–38) and relative humidity (%) (white: < 20; grey: 20–40; light blue: 40–60; blue: 60–80; and dark blue: > 80) bands. Phenological stages (early green tip – EGT, early bloom – EB, and harvest of ‘Duke’ and ‘Brigitta’ are denoted with blue and orange arrows, respectively.